



### Enzyme

One unit of restriction endonuclease completely digests 1 µg of substrate DNA in 1 hour. However, supercoiled plasmid DNA generally requires more than 1 unit/µg to be digested completely. Most researchers add a ten-fold excess of enzyme to their reactions in order to ensure complete cleavage.

**Tip** Ensure that the restriction enzyme does not exceed more than 10% of the total reaction volume, otherwise the glycerol in which the enzyme is supplied may inhibit digestion.

### Reaction volume

Most digests are carried out in a volume between 10 and 50 µl. (Reaction volumes smaller than 10 µl are susceptible to pipetting errors, and are not recommended.)

## Protocol 10. Setting up a restriction digest

## Protocol 10

1. Pipet reaction components into a tube and mix well by pipetting.

**Tip** Thorough mixing is extremely important.

**Tip** The enzyme should be kept on ice and added last.

**Tip** When setting up large numbers of digests, make a reaction master mix consisting of water, buffer, and enzyme, and aliquot this into tubes containing the DNA to be digested.

2. Centrifuge the tube briefly to collect the liquid at the bottom.
3. Incubate the digest in a water bath or heating block, usually for 1–4 h at 37°C. However, some restriction enzymes require higher (e.g., 50–65°C) while others require lower (e.g., 25°C) incubation temperatures.
4. For some downstream applications it is necessary to heat-inactivate the enzyme after digestion. Heating the reaction to 65°C for 20 min after digestion inactivates the majority of enzymes that have optimal incubation temperature of 37°C.

**Tip** Some restriction enzymes are not fully inactivated by heat treatment. The MinElute Reaction Cleanup Kit provides complete removal of restriction enzymes and salts following digestion.

## Ligation of DNA

In order to construct new DNA molecules, DNA must first be digested using restriction endonucleases (see "Restriction Endonuclease Digestion of DNA", page 18). The individual components of the desired DNA molecule are purified and then combined and treated with DNA ligase. The products of the ligation mixture are introduced into competent *E. coli* cells and transformants are identified by appropriate genetic selection. Appropriate control ligations should also be performed (See Protocols 1 and 2, pages 2 and 3).

Removal of 5' phosphates from linearized vector DNA can help prevent vector self-ligation and improve ligation efficiency. To remove 5' phosphates from DNA, add calf intestinal phosphate (CIP) buffer and 1 U CIP and incubate for 30–60 minutes at 37°C. Once the reaction is complete, inactivate CIP by heating to 75°C for 15 minutes.



## Protocol 11. Ligation of DNA and subsequent transformation

## Protocol 11

1. A typical ligation reaction is set up as follows:

- ▶ Component DNAs (0.1–5 µg)
- ▶ Ligase buffer
- ▶ 1 µl 10 mM ATP
- ▶ 20–500 U T4 DNA ligase

2. Incubate for 1–24 h at 15°C.

**Tip** Simple ligations with two fragments having 4 bp 3' or 5' overhanging ends require much less ligase than more complex ligations or blunt-end ligations. The quality of the DNA will also affect the amount of ligase needed.

**Tip** Ligation of sticky-ends is usually carried out at 12–15°C to maintain a balance between annealing of the ends and the activity of the enzyme. Higher temperatures make annealing of the ends difficult, while lower temperatures diminish ligase activity.

**Tip** Blunt-end ligations are usually performed at room temperature since annealing is not a factor, though the enzyme is unstable above 30°C. Blunt-end ligations require about 10–100 times more enzyme than sticky-end ligations in order to achieve an equal efficiency.

3. Introduce 1–10 µl of the ligated products into competent *E. coli* cells and select for transformants using the genetic marker present on the vector (for further information, see Protocols 1 and 2, pages 2 and 3).

4. From individual *E. coli* transformants, purify plasmid or phage DNAs by miniprep procedure and determine their structures by restriction mapping.

**Tip** It is highly recommended to include two controls in every transformation experiment:

- ▶ A “mock” transformation without DNA.
- ▶ A transformation reaction with a known amount of closed circular plasmid DNA.

Controls are essential if things go wrong. For example, colonies on plates that receive mock-transformed bacteria may indicate that the medium lacks the correct antibiotic. An absence of colonies on plates receiving bacteria transformed with plasmids under construction can only be interpreted if a positive control using a standard DNA has been included. See page 4 for further information on transformation controls.

QIAGEN offers a wide range of products for the preparation and isolation of plasmid DNA, DNA cleanup, and PCR fragment cloning (including competent cells), for all throughput and purity requirements. For further information about QIAGEN products and literature please refer to the *QIAGEN Product Guide*, visit us online at [www.qiagen.com](http://www.qiagen.com), or contact QIAGEN Technical Services or your local distributor.

**References**

1. Sambrook, J. and Russell, D. (2001) *Molecular Cloning: A Laboratory Manual*. 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
2. Ausubel, F.M. et al., eds. (1999) *Current Protocols in Molecular Biology*, New York: John Wiley and Sons.
3. Birnboim, H.C., and Doly, J. (1979) A rapid alkaline lysis procedure for screening recombinant plasmid DNA. *Nucl. Acids. Res.* **7**, 1513.
4. Birnboim, H.C. (1983) A rapid alkaline extraction method for the isolation of plasmid DNA. *Methods Enzymol.* **100**, 243.
5. Quantitation of DNA. *QIAGEN News* 1998 No. 2, 23.
6. Effect of pH and ionic strength on the spectroscopic assessment of nucleic acid purity. (1997) *BioTechniques* **22**, 474.
7. QIAprep Miniprep Handbook, March 2001.
8. QIAGEN Plasmid Purification Handbook, September 2000.
9. QIAGEN Plasmid Mini Handbook, March 1999.